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Serum Concentrations of Follicle Stimulating Hormone and Luteinizing Hormone in Laboratory Populations of the Prairie Deermouse (Peromyscus maniculatus Bairdii)

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SERUM CONCENTRATIONS OF FOLLICLE STIMULATING HORMONE AND LUTEINIZING HORMONE IN LABORATORY POPULATIONS OF THE PRAIRIE DEERMOUSE (*PEROMYSCUS MANICULATUS BAIRDII*)

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by

Larry Stephen Hirsch

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APPROVAL SHEET

This thesis is submitted in partial fulfillment

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DEDICATION

This thesis is respectfully dedicated to my grandfather,

Reverend Henry Hirsch.

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ABSTRACT

Radioimmunoassay methods for measuring follicle stimulating hormone (FSH) and luteinizing hormone (LH) in prairie deermice were established and validated. Body weights, paired ovary weights, uterus weights, and serum FSH concentrations were studied for comparisons between nulliparous females from experimental laboratory populations and control females at various stages of the estrus cycle. Body and reproductive organ weights of nulliparous population females were significantly lighter than those of control females. Serum FSH concentrations of nulliparous population females were not significantly different from concentrations of control females. These data indicate that the reproductive inhibition manifest in these nulliparous population female deermice is controlled through components of the hypothalamic-hypophysial-gonadal axis other than FSH. This study suggests that LH may be one factor responsible for the reproductive-endocrine changes observed during curtailed growth. The control females exhibited cyclic changes in both the uterus weights and the serum FSH concentrations that were related to the estrus cycle. The mean serum FSH concentration for nulliparous population females was only significantly different from the mean concentration observed at diestrus.

Body weights, paired testis weights, paired vesicular gland weights, and serum LH concentrations were studied for comparisons between the reproductive-endocrine states of males from experimental laboratory populations and control males. Body and reproductive organ weights of population males were significantly lighter than those of control males. Serum LH concentrations of population males were significantly lower than control male concentrations. These data indicate that an inordinate decrease in circulating LH may be a mechanism whereby reproductive function is curtailed in population males. These data suggest that the drop in circulating androgens, probably the result of altered LH levels, may play an important role in the physiological manifestations observed in inhibited population males.

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INTRODUCTION

When laboratory populations of prairie deermice, Peromyscus maniculatus bairdii, are maintained under conditions of excess food and water, they do not continue to increase in size indefinitely. Rather, each reaches an asymptote (population growth ceases). The numbers of animals present when growth ceases vary widely among the populations even though the conditions of their physical environment are identical. This control of population growth is achieved by one of two mechanisms, either by complete cessation of reproduction, or by failure of the young to survive (Terman, 1965).

Although there is numerical variability at asymptote, the physiological alterations that are manifest during curtailed growth are similar among the populations. Eighty to ninety percent of the females born into the populations and surviving a minimum of ninety days fail to produce progeny (Terman, 1965; 1973a). Reproductive organs of male and females from these populations are significantly lighter in weight than those of control bisexual pairs (Terman, 1969). These findings emphasize the importance of inhibition of reproductive function and maturation in the regulation of population growth. Further, these data suggest that the

mechanisms regulating population growth are triggered by behavioral variables developed intrinsically in each population. These variables are related to behavior-density relationships but are not directly correlated with density *per se* (Terman, 1974; 1975). An animal's response to these variables may possibly be mediated through the hypothalamic-hypophysial-gonadal axis resulting in an inhibition of reproductive function and maturation on the organismal level, thereby yielding curtailment of growth on the population level.

The physiological interrelationships between hypothalamic, hypophysial and gonadal functions in the regulation of population growth remain a matter of considerable controversy. However, studies on individual animals show that under certain conditions the circulating levels of some hormones can change. In 1950, Selye summarized the observable manifestations of physiological response to systemic stress (e.g., muscular exercise, qualitatively or quantitatively inadequate diets, toxic doses of various drugs), and included gonadal atrophy as one of the characteristic changes. He suggested that a reciprocal relationship might exist between the formation and secretion rates of adrenocorticotropic hormone (ACTH) and the gonadotropins. Recent investigations indicate that in laboratory mice and rats various environmental stresses can alter circulating levels of follicle stimulating hormone (FSH) (Ajika *et al.*, 1972; Bronson *et al.*, 1973), luteinizing hormone (LH) (Dunn *et al.*, 1972; Bronson

et al., 1973; Seyler and Reichlin, 1973; Euker et al., 1975), and prolactin (PRL) (Neill, 1970; Ajika et al., 1972; Terbel et al., 1972; Euker et al., 1975). The exact mechanisms by which such "stress" affects the reproductive-endocrine system have not yet been clarified. However, there are indications that stress induced by either ether or pentobarbital treatment in the rat may possibly affect hypophysiotropic hormone activity (Wuttke et al., 1970).

The gonadotropins FSH, LH and PRL are responsible for the attainment and maintainance of reproductive function in both the male and female mouse. In the female, the primary function of FSH is to stimulate young ovarian follicle development (Greep et al., 1942), while LH causes ovulation, stimulates progesterone production and secretion, and acts synergistically with FSH to promote the secretion of estrogen (Greep, 1961). PRL in the female mouse is involved in mammary function (Cowie, 1966) and luteotrophic activity (White and Browning, 1962). These three gonadotropins follow a cyclic mode of secretion from the adenohypophysis in the female. In contrast, the male exhibits a tonic secretion of these gonadotropins. LH activates and stimulates interstitial cells of the testes for androgen production. FSH, in conjunction with the androgens, stimulates sperm production in the seminiferous tubules (Steinberger, 1971). PRL may also play a role in male fertility (Bartke, 1966), though it is not clearly understood.

Until recently, measurement of these gonadotropins involved the use of indirect methods, FSH being measured by the HCG Augmentation bioassay (Steelman and Pohley, 1953), and LH by the Ovarian Ascorbic Acid Depletion bioassay (Parlow, 1961). These assays, however, lack the sensitivity to permit precise determinations of the physiologic plasma concentrations of the glycoprotein hormones in limited quantities of blood. Berson and Yalow (1959) revolutionized the measurement of the polypeptide hormones by describing the new technique of radioimmunoassay (RIA). The principle of the RIA is based on a competition between an endogenous hormone to be measured and a known quantity of radioactively labelled hormone for the sites of a specific antibody added in limited quantity (Berson and Yalow, 1959). The advantage of the RIA is its ability to accurately determine minute hormone concentrations from limited quantities of plasma or serum. The development of the RIA has allowed precise measurements of serum FSH and LH throughout the reproductive cycle in the rat (Gay et al., 1970; Daane and Parlow, 1971; Midgley et al., 1971; Nequin and Schwartz, 1973; Taya and Igarashi, 1973; Butcher et al., 1974), mouse (Kovacic and Parlow, 1972; Murr et al., 1973), and hamster (Goldman and Porter, 1970; Blake et al., 1973; Labhsetwar et al., 1973; Bast and Greenwald, 1974; Varuvidhiz and Meites, 1974; Bex and Goldman, 1975).

Since it is well documented that reproductive function is primarily dependent on FSH and LH, it is possible that the inhibition observed in the individuals of laboratory popula-

tions is due to aberrant circulating levels of these gonadotropins. To test this hypothesis, it was necessary to establish and validate RIA methods for measuring these gonadotropins in Peromyscus maniculatus bairdii, and using these techniques, to then compare the reproductive-endocrine states of population and control animals (paired isolates).

MATERIALS AND METHODS

Paired Isolated Animals (Controls)

Adult female deermice were paired with non-sibling males of the same age (60-90 days) in cages (12x26x14 cm) which allowed reception of visual, olfactory, and auditory cues from the male, but prevented tactile stimulation by means of a partition of two layers of hardware cloth 2 cm apart (Albertson et al., 1975). All control females were housed in the no-contact cages with their corresponding males for a minimum of four weeks before vaginal smears were begun. Vaginal smears were taken daily by saline lavage and examined by Wright stained preparations. Criteria as outlined by Clark (1936) were used for identification of the estrus cycle stage. Only those females displaying a 4- or 5-day cycle for at least ten consecutive days were considered cycling. No distinction was made between the two cycle lengths since an individual female often had both 4- and 5-day cycles. Control females were sacrificed either in the morning period (0900 to 1030 hrs) or in the afternoon period (1500 to 1630 hrs). At each sacrifice period, females were sacrificed for each stage of the estrus cycle to keep sample size as equal as possible. Males were sacrificed immediately following their corresponding female partners. Ages for control females ranged

from 100-300 days with a mean of 202±8 days (Mean±SE), while ages for control males ranged from 101-300 days with a mean of 219±7 days (Mean±SE).

Population Animals

All experimental populations were founded by four bi-sexual pairs of non-sibling prairie deermice between 60-100 days of age. The experimental populations had been used in separate experiments prior to their utilization in this experiment. Population one (Pheromone Exp II, Pop #1) occupied a corrugated galvanized pen (diameter 48.26 cm; floor area 1829.22 cm^2) provided with four plastic nest boxes and woodshavings as bedding. The founding females were not pregnant at the initiation of this population. At sacrifice, population one had reached asymptote (Terman, 1965) by failure of the young to survive. Eighty percent of the females born into this population failed to reproduce. The last surviving litter was born 131 days before sacrifice. Population offspring animals within the age range of the control animals (100-300 days) were used in this study. Ages for offspring females ranged from 128-247 days with a mean of 208±12 days (Mean±SE), while offspring males ranged from 131-247 days with a mean of 211±22 days (Mean±SE). All the female offspring were nulliparous and had closed vaginas at sacrifice. Testes were non-scrotal in all the male offspring at sacrifice.

Population two (Pop Cues Exp III, Pop #8) and population three (Pop Cues Exp III, Pop #3) were reared in circular

enclosures of corrugated aluminum (diameter 60.5 in; floor area 20 ft^2) with eight plastic nest boxes and woodshavings as bedding. The founding females were pregnant at the initiation of these populations. The first litter of each pregnant female was removed from the population at 21 days after birth. The second and successive litters remained as part of that population. Both population two and population three were freely-growing at the time of sacrifice. Population offspring animals whose ages ranged between 100-300 days were used in this study. Female offspring ages for population two ranged from 101-284 days with a mean of 168±19 days (Mean±SE), while ages for population three female offspring ranged from 124-269 days with a mean of 212±18 days (Mean±SE). Male offspring ages for population two ranged from 107-284 days with a mean of 166±13 days (Mean±SE), while population three males ranged from 124-300 days with a mean of 225±13 days (Mean±SE). All the female offspring from population two and population three were nulliparous and had closed vaginas at sacrifice, while 76.1 percent of population two male offspring and 57.1 percent of population three male offspring exhibited the nonscrotal condition of the testes.

Gonadectomized Animals

Thirty male and thirty female deermice (90-100 days of age) were gonadectomized under ethyl ether anesthesia. On postoperative day 35, all animals were sacrificed. All serum samples were collected into one pooled sera designated PCS1.

Animal Care

All animals were housed in 15 x 15 ft rooms maintained under artificial lighting programmed for 11 3/4 hours of bright light (four 40 watt fluorescent tubes) followed by 15 minutes of darkness and then 11 3/4 hours of dim light (four 15 watt incandescent bulbs). The bright light portion for control animals, gonadectomized animals, and animals from populations one and two, was between 0730-1930 hours. Both population one and population two were sacrificed during the afternoon period (1400-1630 hrs) of their light regimen. Population three was sacrificed during the morning period (0130-0330 hrs) of its light regimen (0001-1200 hrs). Temperature in the experimental rooms ranged from 21-31°C, while ventilation was regulated at a complete air exchange every 10 hours. Food and water were supplied ad libitum, and woodshavings used as bedding were changed fortnightly.

Tissue Collection

Blood samples were obtained by anesthetization with ethyl ether and exsanguination via venipuncture of the ascending vena cava at the level of the renal vein using an unheparinized syringe. The total time between the initial contact of an animal and complete collection of a blood sample did not exceed 4 minutes. Blood samples were allowed to clot at 4°C for 8 hours and then centrifuged at 6000 x g, 4°C for 15 minutes. The serum was drawn off and frozen at below -20°C until assayed for FSH or LH. Immediately following blood collection, each animal was weighed and placed with both

the thoracic and abdominal cavities opened in 10 percent formalin. Later, organs were removed from the carcasses, cleaned of fat, lightly blotted and weighed as paired organs to the nearest 0.1 mg.

Materials

All hormones and anti-hormone sera were provided by the Rat Pituitary Hormone Distribution Program, National Institute of Arthritis and Metabolic Diseases (NIAMD), National Institutes of Health. NIAMD-Rat FSH-I-3 (with a biological potency of 1.0 x NIH-FSH-S1), and NIAMD-Rat LH-I-3 (with a biological potency of 1.0 x NIH-LH-S1) were radioiodinated. NIAMD-Rat FSH-RP-1 (with a biological potency of 2.1 x NIH-FSH-S1) and NIAMD-Rat LH-RP-1 (with a biological potency of 0.03 x NIH-LH-S1) served as reference hormones. NIAMD-Anti-Rat FSH Serum-6 and NIAMD-Anti-Rat LH Serum-1 were the first antibodies in the appropriate double-antibody radioimmunoassay.

Goat anti-rabbit gamma globulin, used as the secondary antibody in both assays, was purchased from Antibodies, Incorporated (Davis, California).

Radioiodination

Purified rat FSH (Rat FSH-I-3) and purified rat LH (Rat LH-I-3) were trace-labelled with ^{131}I at room temperature by a modification of the method of Greenwood et al., (1963), as described by Niswender et al., (1969). Twenty-five ul of 0.5 M phosphate buffer, pH 7.5, was mixed at the bottom of a

1 ml serum vial with 2.5 ug of the purified hormone previously diluted to 0.1 ug/ul in 0.01 M phosphate, 0.14 NaCl, pH 7.0, (PBS). One mCi of thiosulfate-free iodide ($Na^{131}I$) in 0.1 N NaOH (Cambridge Nuclear Corporation) was added to the vial and its contents were gently mixed. Twenty ug of chloramine-T in 25 ul of 0.05 M phosphate buffer, pH 7.5, was added and a stopper inserted. The reaction mixture was then agitated for exactly two minutes by gently finger tapping the vial. The reaction was stopped by adding 120 ug of sodium metabisulfite in 50 ul of 0.05 M phosphate buffer. Transfer solution (100 ul) containing 16 percent sucrose and 1 mg KI was added and the contents of the vial were layered beneath the buffer on a 0.7 x 10 cm column of Biogel P-60 (Bio-Rad Labs) equilibrated with 0.05 M phosphate buffer. The column was eluted with the equilibration buffer as aliquots (7 drops) of the eluate were collected in tubes containing 0.5 ml of PBS-5%eggwhite (PBS-EW). The samples of eluate were then counted in a lead pig centered on the top of a Tracerlab 132M manual well-type gamma scintillation counter with a 3 inch crystal adjusted for measuring the 0.364 Mev photopeak of ^{131}I. The leading fraction of the protein peak, usually tube 4 or 5, was immediately diluted with PBS-0.1%EW so that on the day after preparation, 100 ul of the solution gave from 10,000 to 20,000 cpm. To determine the recovery of the labelled hormone, and the extent of utilization of ^{131}I, all vials, transfer pipettes, stoppers, et cetera, were counted with appropriate corrections made for background and volume effects.

Radioimmunoassay

All hormones were measured by double-antibody radioimmunoassays using the non-equilibrium reaction conditions described by Midgley et al., (1969), following the protocol outlined by Diebel et al., (1973). In 12 x 75 mm disposable reaction tubes, varying amounts of sample or standard were placed with an appropriate amount of PBS-1%EW so that each tube contained a common volume of 500 ul. To each tube was added 200 ul of either FSH or LH antiserum, appropriately diluted (anti-FSH, 1:25,000; anti-LH, 1:100,000) with 1:400 normal rabbit serum so that the 200 ul of antiserum bound 25-30 percent, or 30-35 percent of the radioiodinated FSH or LH, respectively, in an assay tube lacking unlabelled hormone. The tubes were then incubated at 4°C for 36 hours, after which 100 ul of ^{131}I-labelled hormone was added. After an additional 24 hours of incubation at 4°C, 200 ul of goat anti-rabbit gamma globulin was added to each tube at a dilution sufficient to precipitate optimally the rabbit gamma globulin in each tube (usually 1:65). After a further incubation of 3-5 days, 3 ml of cold PBS was added to each tube to dilute the supernatant radioactivity. The precipitate was then separated from the free hormone by centrifugation at 1000 x g, 4°C for 45 minutes. The supernatant was decanted, and the precipitate counted in the well of a Tracer-lab 132M manual well-type gamma scintillation counter for a sufficient time period to minimize the counting error to below two percent (10,000 counts).

Hormone concentrations were calculated from the linear regression equation following the transformation of the standard curve data to log dose and logit response utilizing a computer analysis program (Duddleson et al., 1971). Hormone concentrations are expressed in terms of NIH FSH-RP-1 and NIH LH-RP-1. Concentrations lower than the limits of sensitivity were given those values in calculating means and standard errors, following the method of Murr et al., (1971).

Protocol

After all serum samples had been collected, they were thawed once and assayed for either FSH or LH. Male animals were assayed for LH while female animals were assayed for FSH. One hundred ul of serum from each animal was assayed either in duplicate or as a single determination, depending on the total volume obtained during exsanguination. Multiple-dose levels of pooled sera (PCS1) were assayed concurrently with the sera from the control and population animals. The sera were run in two radioimmunoassays. Serum samples from all stages of the estrus cycle and from all population groups were included in each assay.

Statistics

The slopes of the standard curves from the two RIAs for each hormone were compared for similarity according to the analysis described by Snedcor and Cochran (1967). Comparisons of body weights, paired testis weights, paired vesicular gland weights, serum LH concentrations, paired ovary weights,

uterus weights, and serum FSH concentrations were made using the means of the experimental populations, because the individuals within each experimental population are not statistically independent. Control and experimental population data were compared using the Mann-Whitney U test (Conover, 1971). For uterus weights and serum FSH concentrations, a posteriori comparisons were made between the various stages of the estrus cycle and population females utilizing analysis of variance and Duncan's multiple range test (Steel and Torrie, 1960). Correlations were made using Pearson's product-moment method. Data were also subjected to linear regression analysis (Steel and Torrie, 1960; Searle, 1971). The five percent level of probability was regarded as significant in all cases.

RESULTS

Iodination

A typical elution pattern from a Biogel P-60 column showing the separation of FSH-^{131}I and free ^{131}I is shown in figure 1. Figure 2 shows a typical elution pattern of radioiodinated LH. The difference in the degree of iodination between the two gonadotropins is very pronounced in these figures, with FSH having the greater uptake of the nuclide. For both FSH and LH, the first peak to emerge (usually tube 3 or 4) contained the labelled glycoprotein. The second peak was mostly free iodide. When an aliquot of the first peak from either FSH or LH was passed through a 1 x 30 column of Sephadex G-100, it separated into three peaks. Similar experiments have shown that the first peak (about 15%) is aggregated gonadotropin, which is very poorly immunoreactive. The second and largest peak (about 80%) consists of the immunoreactive material, whereas the third and smallest peak is presumably iodide (Sinha *et al.*, 1972). Utilization of the ^{131}I for FSH ranged from 24.6-70.4 percent while LH utilization of ^{131}I ranged from 11.8-69.2 percent.

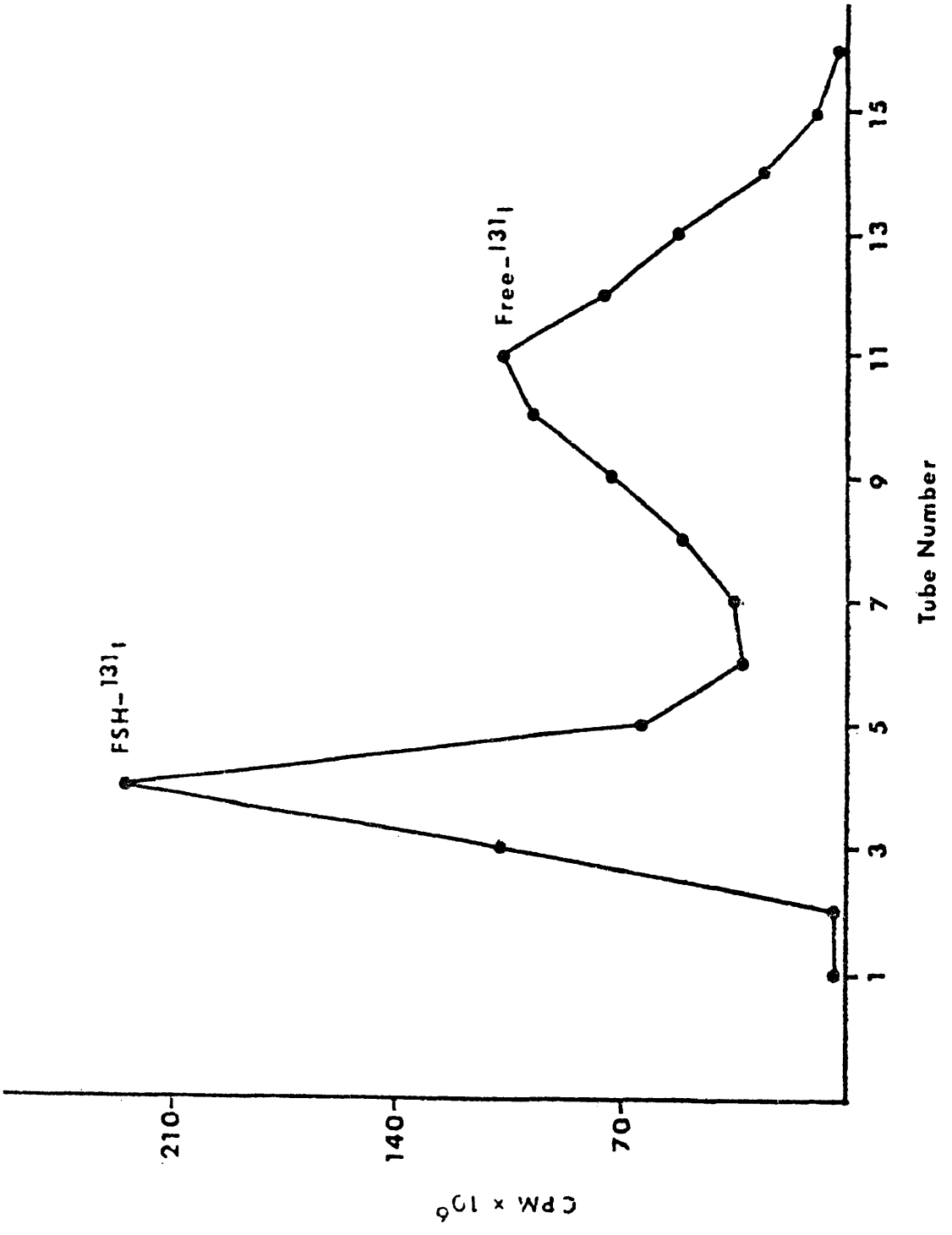
Assay Characteristics

Figure 3 and figure 4 show typical standard curves for

Legend for Figure 1

Elution profile of FSH iodination reaction mixture from a 0.7 x 10 cm column of Biogel P-60. Each point represents the radioactivity in successive seven drop aliquots.

Figure 1


FSH-^{131}I
Free-^{131}I
CPM x 10^6
210
140
70
1
3
5
7
9
11
13
15
Tube Number

Legend for Figure 2

Elution profile of LH iodination reaction mixture from a 0.7 x 10 cm column of Biogel P-60. Each point represents the radioactivity in successive seven drop aliquots.

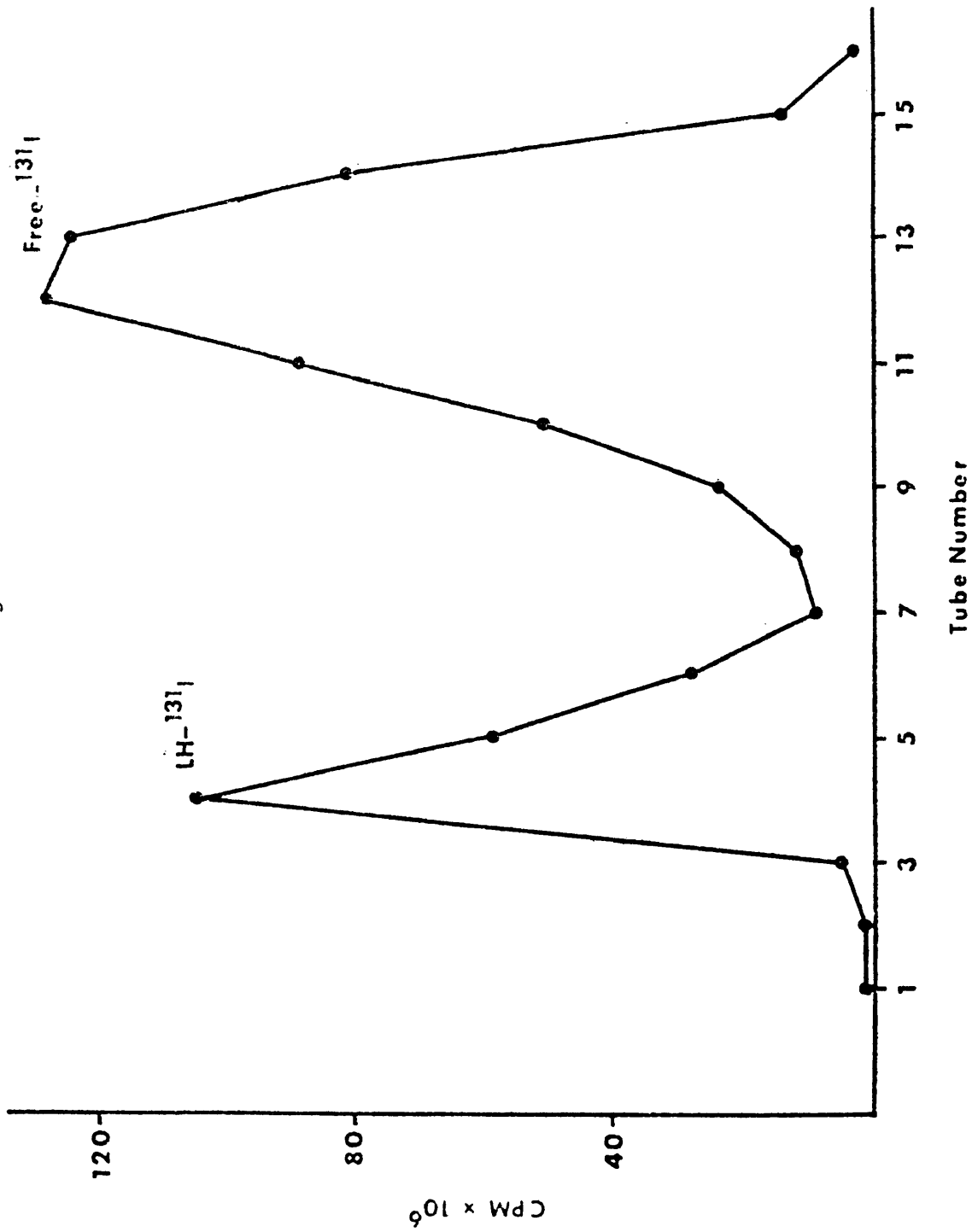


Figure 2

FSH and LH, respectively. The FSH standard curve ranged from 0-1000 ng, whereas the LH standard curve ranged from 0-100 ng. If 100 ul of a serum sample is used for either assay, the FSH system can detect as little as 31.5 ng of FSH/ml of serum, while the LH system can detect as little as 1.2 ng of LH/ml of serum.

The dose-response curves for the Peromyscus castrate sera pool (PCS1) are shown with the NIH rat standard curves in figures 3 and 4. The slope of the regression line for the FSH reference hormone was not significantly different from the slope of the line for PCS1 in three radioimmunoassays. However, the slope of the regression line for the LH reference hormone was significantly different ($P<.05$) from the slope of the line for PCS1 in three radioimmunoassays. This necessitated the use of the PCS1 dose-response curve as the reference standard curve for LH concentration calculations. The concentration of hormone in the Peromyscus castrate sera pool (PCS1) was 2470±102 ng FSH/ml (Mean±SE, 3 assays) and 168±12 ng LH/ml (Mean±SE, 3 assays), when assayed at 9-10 multiple-dose levels per assay. The reference curves from the two radioimmunoassays were not significantly different for either hormone, and the data from the two separate determinations were, therefore, pooled.

Control Estrus Cycle

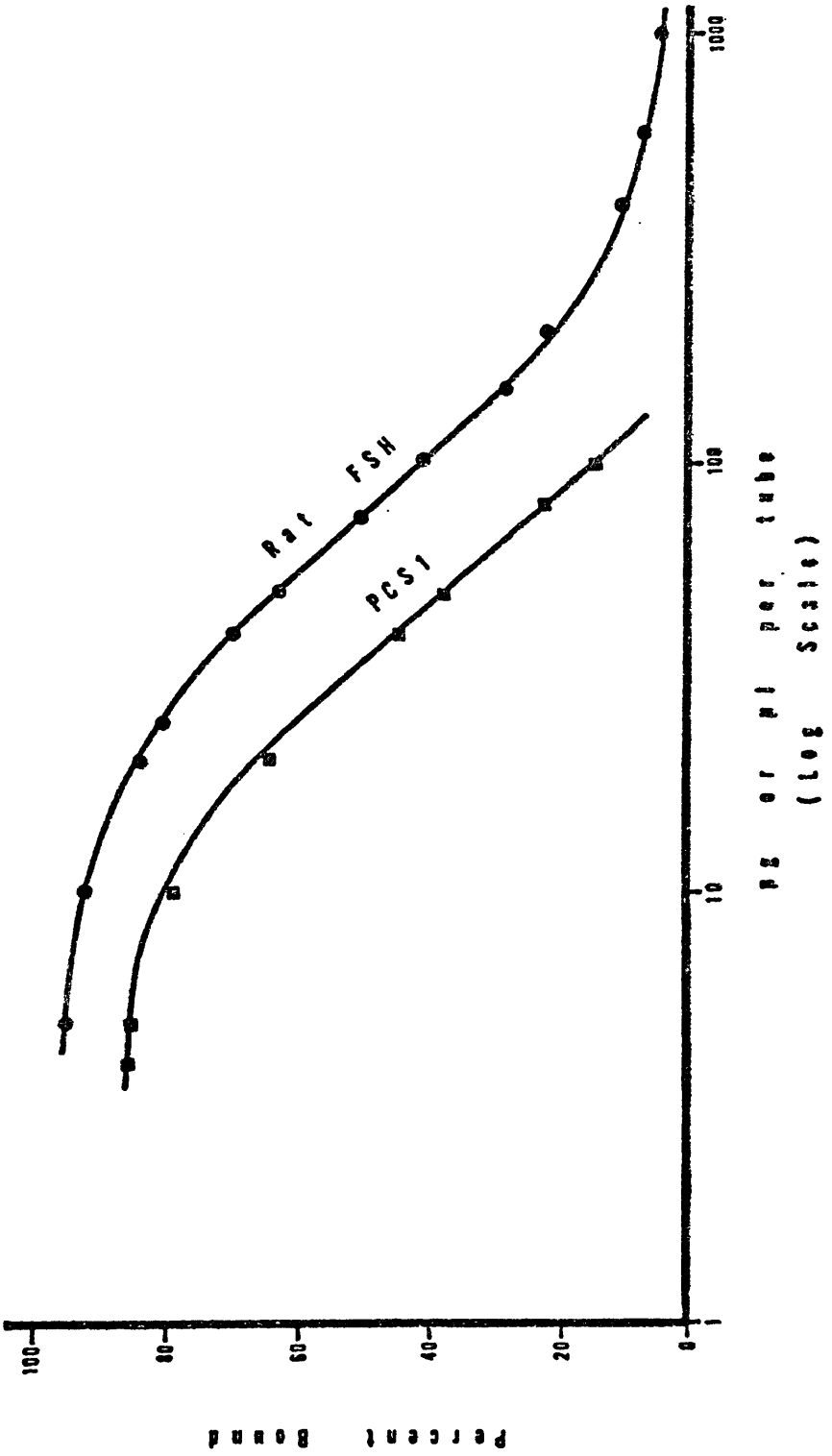
Serum FSH Concentrations (Table 1, Figure 5)

Cyclic variations in the serum FSH concentrations were observed throughout the stages of the estrus cycle (Appendices

Legend for Figure 3

Radioimmunoassay standard curve for NIAMD rat FSH reference preparation (NIAMD-rat FSH-RP-1) with the dose-response curve for Peromyscus castrate sera pool (PCS1). NIAMD rat FSH curve is in ng/tube and PCS1 is in ul/tube.

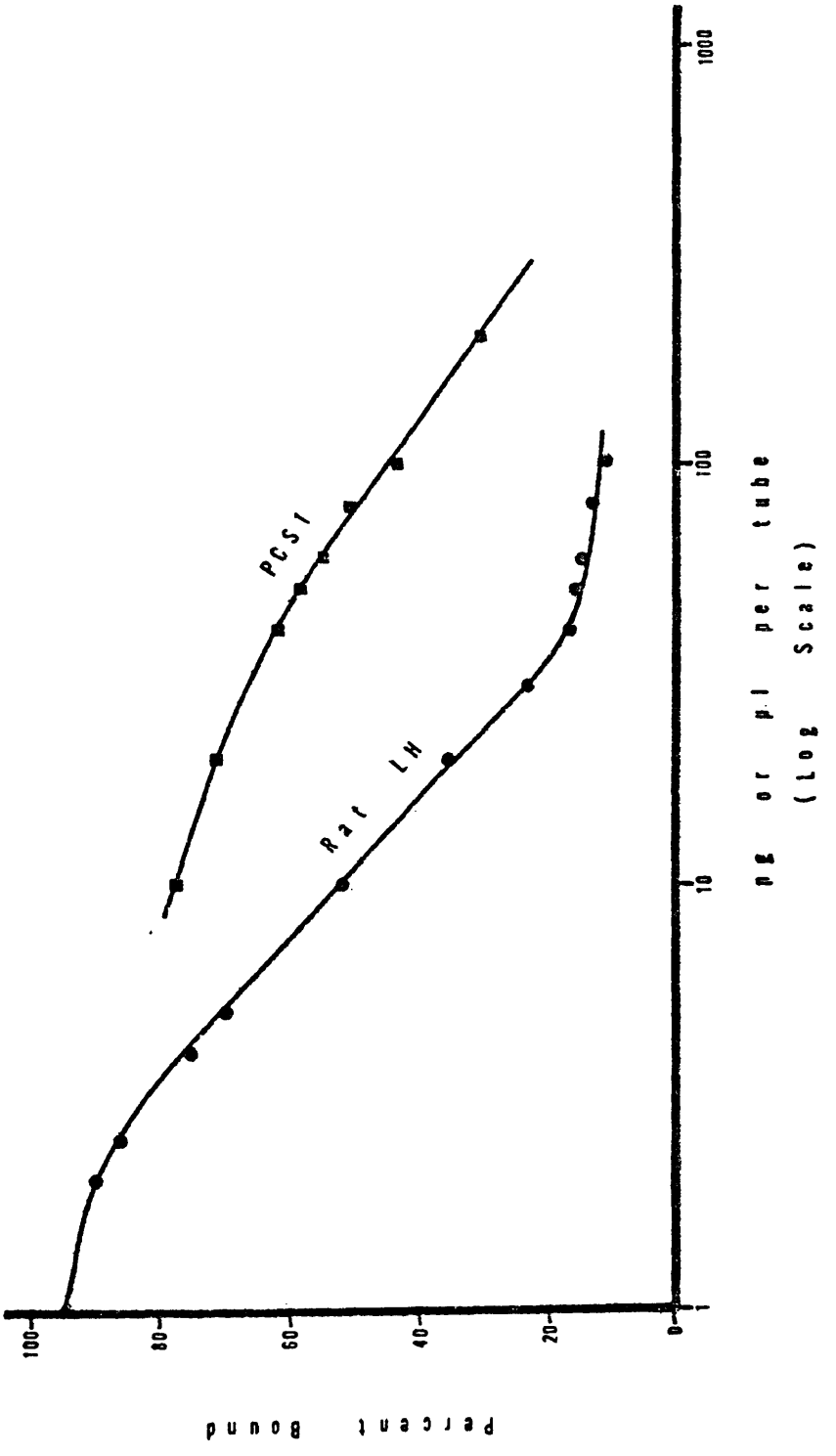
Figure 3


Percent Bound
100
80
60
40
20
0
1
10
100
1000
ng or µl per tube
(Log Scale)
Rat FSH
PCS1

Legend for Figure 4

Radioimmunoassay standard curve for NIAMD rat LH reference preparation (NIAMD-rat LH-RP-1) with the dose-response curve for Peromyscus castrate sera pool (PCS1). NIAMD rat LH curve is in ng/tube and PCS1 is in ul/tube.

Figure 4


PCSI
Rat LH
100
80
60
40
20
0
1
10
100
1000
pg or µl per tube
(log Scale)
percent bound

E and F). Serum FSH levels, which were low on the day of diestrus, rose slightly on the morning of proestrus. In the afternoon of proestrus, serum FSH rose sharply and remained high on the morning of estrus. Serum FSH concentrations during estrus were not significantly different from the concentrations on proestrus afternoon. Levels of serum FSH decreased gradually following the morning of estrus. When morning period FSH concentrations were compared with their corresponding afternoon FSH concentrations, no significant differences were observed in any of the stages. Therefore, the data (Appendix E) from these two periods were pooled within each of these stages for further comparisons (Table 1).

Paired Ovary Weights (Appendix E)

Analysis of variance indicated that the paired ovary weights were not significantly different as a function of the estrus cycle stage or time period within that stage.

Uterus Weights (Appendix E, Table 2, Figure 5)

No significant differences were observed within stages of the estrus cycle when the uterus weights of animals sacrificed in the morning were compared with those sacrificed in the afternoon (Appendix E). Therefore, the uterus weight data were pooled from the morning and afternoon periods within each stage of the estrus cycle (Table 2). Pooled uterus weights were highest on the day of proestrus and lowest on the day of diestrus (Table 2). There was no significant difference between the mean uterus weight values for proestrus

TABLE 1

Comparisons of Serum Follicle Stimulating Hormone Concentrations (ng/ml)
throughout the Estrus Cycle of Control Deermice
and of Population Nulliparous Females

Serum FSH Concentrations			Duncan's Multiple Range Comparisons			
Control Females	N	Mean ± SE	Estrus	Metestrus	Diestrus	Population Nulliparous Females
Proestrus	(18)	220.1±29.5	N.S.	N.S.	P<.01	N.S.
Estrus	(16)	289.1±34.1	--	P<.01	P<.01	N.S.
Metestrus	(15)	192.1±16.0		--	P<.01	N.S.
Diestrus	(18)	93.7±23.1			--	P<.01
Population Nulliparous Females	[3](26)	270.2±46.7				--

Bracket refers to the number of population means
Parentheses refer to the number of animals contributing to the means
N.S. = P>.1

TABLE 2

Comparisons of Uterine Weights (mg)
throughout the Estrus Cycle of Control Deermice
and of Population Nulliparous Females

	Uterine Weights		Duncan's Multiple Range Comparisons			
Control Females	N	Mean ± SE	Estrus	Metestrus	Diestrus	Population Nulliparous Females
Proestrus	(18)	75.7±6.4	N.S.	P<.01	P<.01	P<.01
Estrus	(16)	67.8±7.4	--	P<.01	P<.01	P<.01
Metestrus	(15)	47.7±7.5		--	N.S.	P<.01
Diestrus	(18)	37.3±2.7			--	P<.01
Population Nulliparous Females	[3](34)	9.2±0.6				

Bracket refers to the number of population means
Parentheses refer to the number of animals contributing to the means
N.S. = P>.1

and estrus, or between the means for diestrus and metestrus. Both proestrus and estrus means for uterus weights were significantly larger ($P<.01$) than the means of diestrus and metestrus.

Correlations and Regressions (Appendices I and J)

A significant and positive correlation ($P=0.0001$; $r=0.5485$) was observed between body weights and paired ovary weights (Appendix I). A significant positive correlation ($P=0.0032$; $r=0.3327$) was also found between body weights and uterus weights.

Comparisons of Control and Population Nulliparous Females (Appendix H)

Body Weights (Table 3)

Nulliparous deermice from experimental populations had significantly lower ($P<.01$) mean body weights than control females.

Paired Ovary Weights (Table 3)

Population nulliparous females had significantly smaller ($P<.01$) mean paired ovary weights than control females. The mean relative paired ovary weights of population females was also significantly lower ($P<.01$) than that of control females.

Uterus Weights (Tables 2 and 3, Figure 5)

The mean uterus weights of population nulliparous females was significantly smaller ($P<.01$) than the mean of control females (Table 3). Moreover, mean uterus weights of population

TABLE 3

Comparisons of the Body Weights (gm), Reproductive Organ Weights (mg), Relative Reproductive Organ Weights (mg/gm) and Serum FSH Concentrations (ng/ml) of Population and Control Female Deermice

	Control		Population (nonparous)		
	N	Mean ± SE	N	Mean ± SE	P
Body Weights	82	16.5± 0.2	3(34)	12.7± 0.2	<.01
Ovaries (paired)	79	15.4± 0.8	3(34)	4.9± 0.3	<.01
Relative Ovaries	76	0.9± 0.1	3(33)	0.4± 0.2	<.01
Uterus	80	55.4± 3.8	3(34)	9.2± 0.6	<.01
Relative Uterus	76	3.2± 0.4	3(34)	0.7± 0.4	<.01
Serum FSH	81	228.8±17.6	3(34)	270.2±46.7	N.S.

N refers to the number of population mean values
Parentheses refer to the number of animals contributing to the means
N.S. = P>.1

Legend for Figure 5

Mean±SE serum concentrations of FSH and Mean±SE uterus weights for the various stages of the control estrus cycle and for population female prairie deermice.

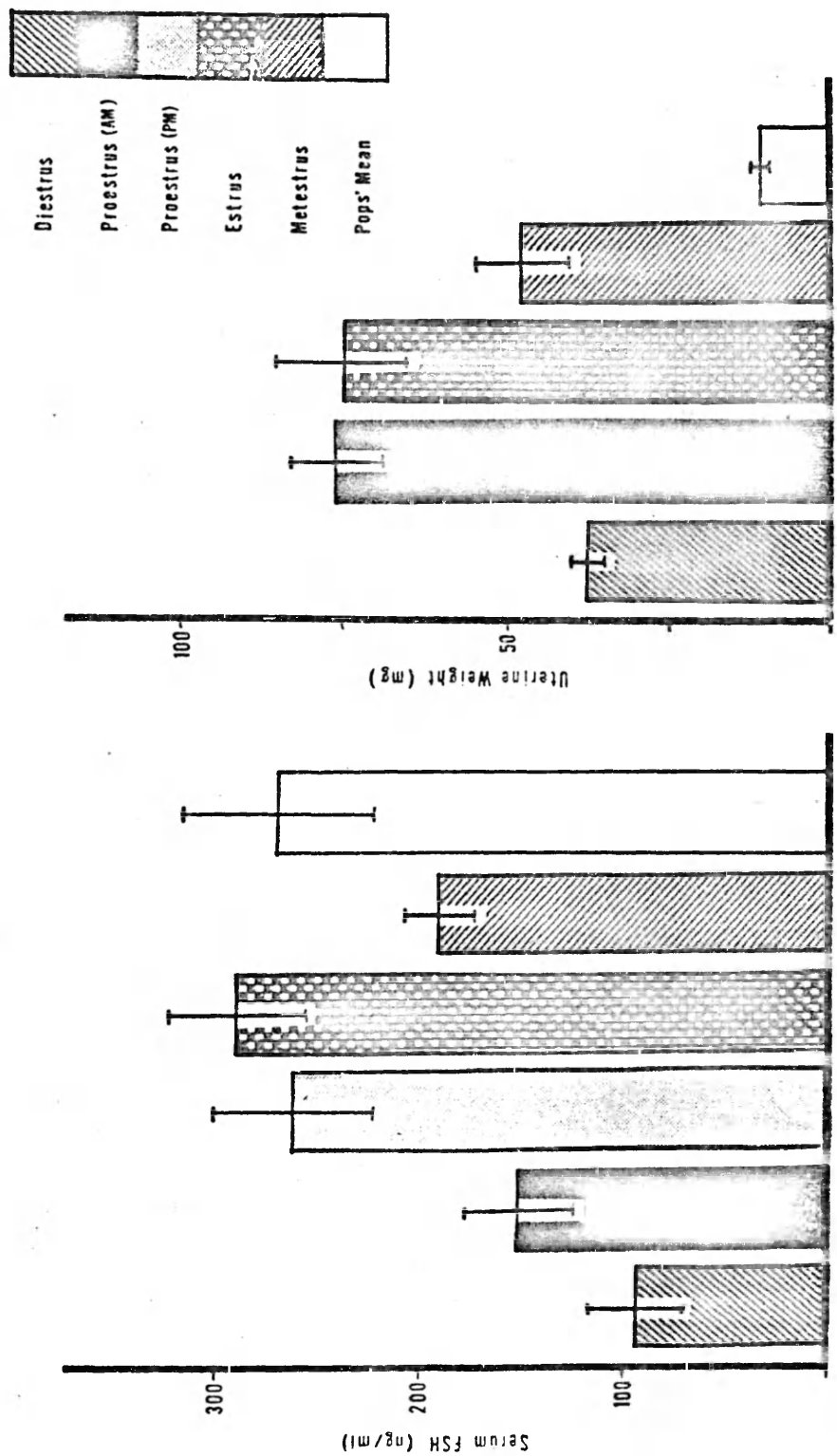

Diestrus
Proestrus (AM)
Proestrus (PM)
Estrus
Metestrus
Pops' Mean
Uterine Weight (mg)
100
50
Serum FSH (ng/ml)
300
200
100

Figure 5

nulliparous females were significantly smaller ($P<.01$) than the mean uterus weights of each stage of the estrus cycle (Table 2). The mean relative uterus weight for nulliparous population females was significantly smaller ($P<.01$) than that of control females (Table 3).

Serum FSH Concentrations (Tables 1 and 3, Figure 5)

No significant difference was observed when the mean serum FSH concentration of nulliparous population females was compared with the mean of control females (Table 3).

Table 1 presents comparisons of serum FSH concentrations in nulliparous population females and control females in specific stages of the estrus cycle. Nulliparous population females had a significantly higher ($P<.01$) mean FSH concentration than control females in diestrus. The mean serum FSH concentration of population nulliparous females was not significantly different than that of control females from proestrus, metestrus and estrus.

Control Males (Appendices A and D, Table 4)

No significant differences were observed between the body weights, paired testis weights, paired vesicular gland weights and serum LH concentrations of males sacrificed in the morning and afternoon periods (Appendix A). Data from the morning and afternoon periods were pooled for further comparisons (Table 4). Significant regressions were observed between age and body weights ($P=0.0031$; $Y=0.01795\ X + 15.301$; $r=0.3089$), and between age and paired vesicular gland weights

TABLE 4

Comparisons of the Body Weights (gm), Reproductive Organ Weights (mg), Relative Reproductive Organ Weights (mg/gm) and Serum LH Concentrations (ng/ml) of Population and Control Male Deermice

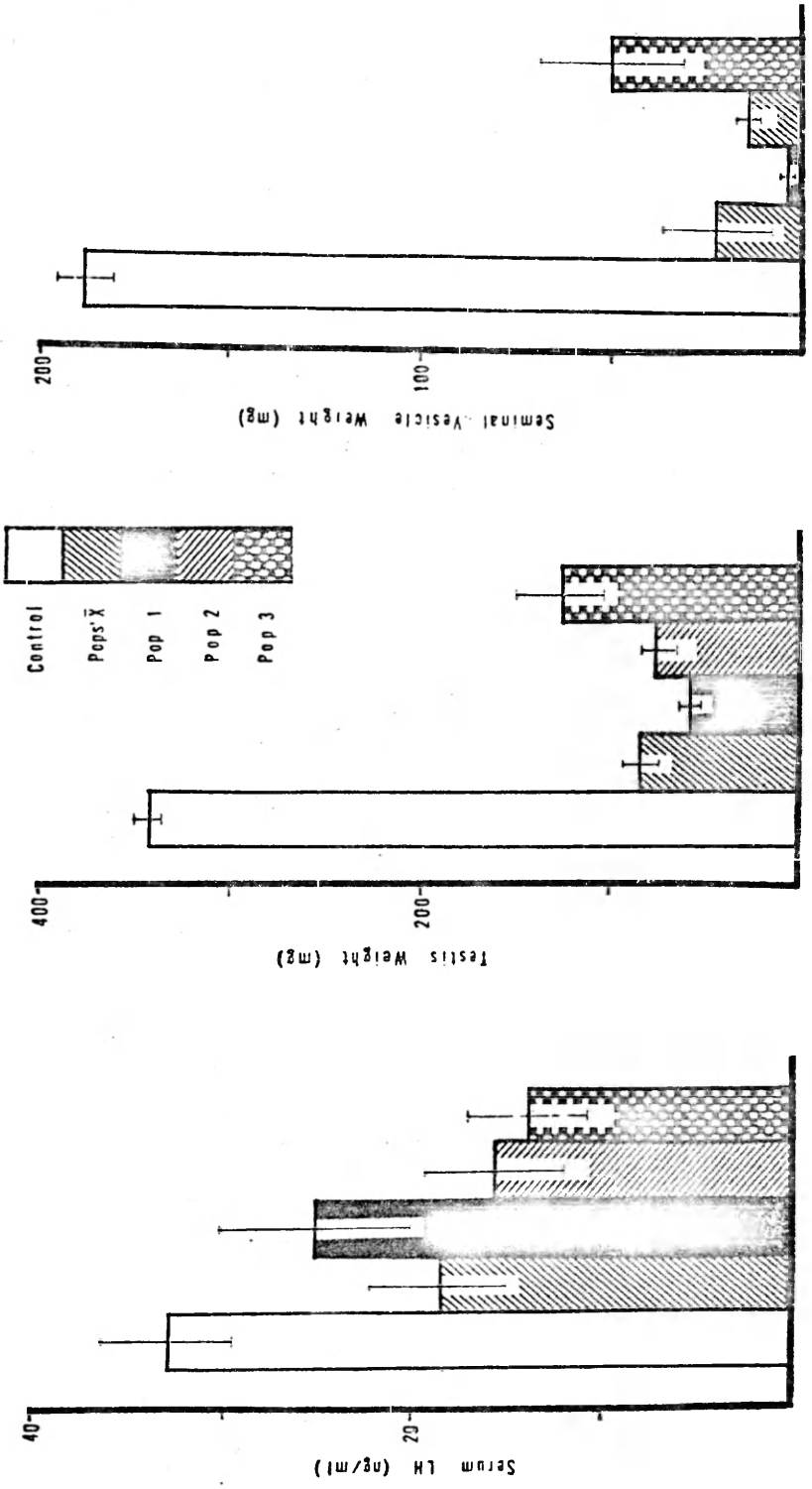
	Control		Population		
	N	Mean ± SE	N	Mean ± SE	P
Body Weights	99	19.2±0.4	3(38)	13.8± 0.2	<.01
Testes (paired)	98	343.2±7.2	3(39)	83.1±22.5	<.01
Relative Testes	85	18.2±0.4	3(38)	5.9± 1.4	<.01
Vesicular Glands (paired)	95	188.8±6.7	3(40)	22.1±10.4	<.01
Relative Vesicular Glands	85	10.3±0.4	3(38)	1.5± 0.9	<.01
Serum LH	71	33.4±3.7	3(36)	18.9± 3.5	<.1
			(36)*	17.0± 2.3	<.001

N refers to the number of population mean values
Parentheses refer to the number of animals contributing to the means
* refers to comparisons using the mean of individual population animals

Legend for Figure 6

Mean±SE serum concentrations of LH, Mean±SE paired testis weights, and Mean±SE paired seminal vesicle (vesicular gland) weights for control and population male prairie deermice.

Figure 6


Seminal Vesicle Weight (mg)
200
100
Testis Weight (mg)
400
200
Control
Pops X̄
Pop 1
Pop 2
Pop 3
Serum LH (ng/ml)
40
20

(P=0.0019; Y=-0.30104 X + 258.09; r=-0.3254) (Appendix D). A significant and slightly positive correlation (P=0.0106; r=0.2701) was demonstrated between body weights and paired testis weights (Appendix C). A significant positive correation (P=0.0001; r=0.5003) was also demonstrated between paired testis weights and paired vesicular gland weights (Appendix C). The mean serum LH concentration for control male prairie deermice was 33.4±3.7 ng (NIAMD-Rat LH-RP-1) (Table 4). This mean value compares with the mean control value reported for the laboratory mouse (Kovacic and Parlow, 1972) of 115±26 ng (NIAMD-Rat LH-RP-1), and the mean control value reported for the golden hamster (Goldman and Porter, 1970) of 11.5±4.2 ng (NIH-LH-S1) for morning sacrifice, and 11±2.7 ng for afternoon sacrifice.

Comparisons of Control and Population Males (Appendix B)

Body Weights (Table 4, Figure 6)

Males from experimental populations had significantly lower (P<.01) mean body weights than control males.

Paired Testis Weights (Table 4, Figure 6)

The mean paired testis weights of population males was significantly smaller (P<.01) than the mean of control males. Relative paired testis weights of population males were also significantly smaller (P<.01) than those of controls.

Paired Vesicular Gland Weights (Table 4, Figure 6)

The mean paired vesicular gland weights of population males was significantly smaller (P<.01) than the mean of

control males. The mean relative paired vesicular gland weights of population males was also significantly smaller ($P<.01$) than that of controls.

Serum LH Concentrations (Table 4, Figure 6)

A difference at only $P<.1$ was found between the populations' mean serum LH concentration and the mean of control males. However, when population males were treated as statistically independent individuals, their pooled serum LH concentrations mean was significantly lower ($P<.01$) than the mean of control males.

DISCUSSION

Females

This part of the study documents the sequence of changes in serum FSH during the estrus cycle of control Peromyscus (Table 1, Figure 5). The single protracted FSH peak observed in the prairie deermouse is similar to that described for the laboratory mouse (Kovacic and Parlow, 1972; Murr et al., 1973) and laboratory rat (Daane and Parlow, 1971; Taya and Igarashi, 1973). The surge of FSH during proestrus is thought to stimulate growth of the crop of follicles which will ovulate during the succeeding estrus cycle (Murr et al., 1973).

Uterine weights (Table 2, Figure 5), which were highest on the day of proestrus, are in agreement with other reported observations in mice (Bingel and Schwartz, 1969; Desjardins et al., 1970; Kovacic and Parlow, 1972). Such a response is expected since it is well established that one effect of the estrogens secreted from the ovary is to cause the accumulation of fluids in the uterus during proestrus. Although various roles for this fluid increase have been suggested, such as facilitation of sperm transport (Warren, 1936; Leonard, 1950) and participation in sperm capacitation (Noyes, 1953; Kirton and Hafs, 1965), the exact physiological sig-

nificance remains to be established (Armstrong, 1968). Uterine weights for laboratory mice have been reported by Kovacic et al., (1972), and Desjardins et al., (1970), to be low in metestrus, while Bingel and Schwartz, (1969), have reported them low in diestrus I. The present study indicates that deermice uterine weights were still declining in metestrus and reached a nadir in diestrus (Table 2, Figure 5). In the rat the sudden loss of uterine lumen fluid, which normally occurs late in estrus, is primarily the result of increased synthesis and secretion of progesterone in response to luteinizing hormone (Armstrong, 1968). The rather protracted peak of uterine weights in estrus and the latent period of decline over metestrus and diestrus observed in this study may be explained by examining progesterone serum levels reported throughout the deermouse estrus cycle by Albertson et al., (1975). Deermouse mean plasma progesterone concentrations increased 103 percent from proestrus to estrus, reached their highest levels at metestrus, which was a 177 percent increase over estrus, and then declined in diestrus, decreasing 52 percent from the metestrus high (Albertson et al., 1975). The present study found that uterine weights declined significantly between metestrus and diestrus, during which time the previous study (Albertson et al., 1975) indicated a 52 percent decline in progesterone. Thus, the uterine weights seem clearly dependent upon progesterone concentrations. The uterus in Peromyscus seems to manifest changes typical of a biphasic ovarian cycle.

Comparisons between the reproductive organ weights of control and experimental population females indicate the drastic nature of inhibition of reproductive function among population animals. The mean paired ovary weights of nulliparous population females (Table 3) was less than one-third the weight of controls, while their mean uterus weights (Table 3) was only seventeen percent of the control uterus weights. Both the mean relative paired ovary weights and the mean relative uterus weights of nulliparous population females were also significantly lower than those of controls (Table 3). Therefore, the smaller ovaries and uterus conditions found in these population females cannot be solely attributed to their smaller body weight condition.

Especially striking is the high serum FSH level observed in these nulliparous population females, which is not out of the range observed in cycling control females (Tables 1 and 3, Figure 5). This suggests that the reproductive inhibition observed in these female deermice is controlled by other components of the hypothalamic-hypophysial-gonadal axis. Both the closed vagina and the abnormally small uterus weight (Tables 2 and 3) conditions found in these animals are indicative of little, if any, estrogen secretion (Lloyd et al., 1946; Schwartz, 1964). These data concur with those previously reported (Terman, 1965; Albertson et al., 1975), and with the notion that with respect to estrogens, nulliparous population females are not cycling (Albertson et al., 1975). Thus, the FSH level found in these nulliparous population fe-

males may be the result of an insufficient feedback signal from the estrogens to the hypophysis or higher centers (Bogdanove, 1963; Schwartz, 1969; Gay, 1972). This is supported by the evidence that the mean serum FSH concentration of nine acyclic control females was also elevated (Appendix G). In fact, five of these acyclic paired isolated females had serum FSH concentrations which were out of the range of cycling paired isolated females. Therefore, it appears that a range of inhibition may exist with acyclic females being the most depressed followed by inhibited population females. The higher FSH concentration found in these acyclic females, when compared with population females, may be due to a permanent physiologic dysfunction condition in acyclic females, while the inhibition in experimental population females is apparently a temporary condition.

Experimental evidence from several mammalian species indicate that cyclic stimulation of the hypophysis is inherent in the female hypothalamus. However, it is increasingly evident that it is the changing input from the ovarian secretions that determines the cyclic secretion of the hypothalamus and the hypophysis rather than a rhythm inherent in the axis. Both FSH and LH are subjected to negative feedback mechanisms by the steroids. This is evident from other studies which have shown a rise in FSH and LH after castration or during the menopause, and when these high levels are depressed after administration of estrogens. In addition to a negative feedback upon FSH and LH secretion, gonadal

hormones also exert a positive effect on gonadotropin secretion. Evidence suggests that it is the changing and rising titer of estrogens that stimulates the LH surge. However, estrogens do not appear to have a positive feedback effect upon FSH secretion. It can be assumed that positive and negative mechanisms operate simultaneously and that the net change in the secretion of the gonadotropins represents the algebraic sum of the positive and negative feedback inputs (Schwartz and McCormack, 1972; Ross and Vande Wiele, 1974).

Follicle maturation is dependent upon the stimulatory effect of both FSH and LH in appropriate ratios. FSH is involved in antrum formation and the growth of primary follicles (Greep et al., 1942). The role of FSH in the production of ovarian estrogen appears to be its effect on providing an abundance of developed follicles (together with minimal quantities of LH) that are capable of being stimulated by LH to secrete steroids (Stevens, 1972). FSH in some way prepares the follicle for the action of LH and enhances the release of estrogen induced by LH, although there is no good evidence that FSH per se stimulates steroid production in the ovary (Villee, 1975). While tonic discharge of LH and FSH is thought to maintain estrogen secretion, it is the surgelike rise in secretion of LH that leads to final steps in maturation of the graafian follicle, follicle rupture, and expulsion of the oocyte (Odell and Moyer, 1971; Ross, 1974).

Although the present data do not reveal a chronic level of FSH that might result in the drastic changes observed in

the reproductive function of these females, the data do raise the question of whether or not the ovary is refractory to the presence of FSH. The important ovarian histology which would help clarify this question was not done in the present study. However, if it is assumed that the ovarian histology and serum progesterone levels in the nulliparous females from the present study are similar to those reported for females in earlier studies (Terman, 1973b; Albertson et al., 1975), then this evidence may be interpreted as arguments against the notion that the ovaries in these females are refractory to FSH. Specifically, one study shows that the serum progesterone levels of nulliparous population females are within the range of cycling control females (Albertson et al., 1975). These data may indicate, at least with respect to progesterone production and secretion, that the ovaries are able to respond to gonadotropin stimulus. The Albertson et al. study also reported that in all the populations examined all stages of follicular development were observed, although there were significantly fewer follicles when compared to controls, and that 77 percent of the ovaries contained corpora lutea. These data not only suggest that the gonadotropins are being released in some of these females, but also suggest that the ovaries are able to respond to gonadotropin stimulus with production and growth of follicles, and perhaps even with ovulation.

Arguments in support of the notion that the ovaries are refractory to FSH, and possibly to LH, arise from the indirect

evidence that estrogen levels are inordinately reduced in these animals. Since both FSH and LH are necessary for ovarian estrogen secretion (Lostroh and Johnson, 1966), it could be that the ovaries are refractory to both FSH and LH, resulting in curtailed estrogen production and secretion. However, ovarian histology observed in other nulliparous population females showed that these animals had fewer follicles of maximum size when compared with controls (Terman 1973b; Albertson et al., 1975). Thus, if the ovarian histology of the females from the present study is similar to that observed in females from these other studies, then the ovarian histology data may indicate that primary follicles are stimulated by FSH to develop up to the stages when other factors, such as LH, are required for final maturation (Stevens, 1972). This view would suggest that the ovaries are responsive to FSH but that LH is either not present or below the threshold concentration required for its ovarian action. Although LH is usually the gonadotropin responsible for eliciting progesterone secretion, high FSH can cause increased progesterone secretion and luteinization of follicles (Schwartz and Ely, 1970; MacDonald, 1971), which could account for progesterone secretion and luteinization of some of the unovulated follicles (Albertson et al., 1975). Whether or not the reproductive changes observed in the females are the result of a temporary refractory state of the ovaries to one or both of the gonadotropins remains unresolved at the present.

It may be that the reproductive inhibition observed in these females is due to aberrant changes in the circulating levels of LH. Possibly LH is circulating at levels below the threshold concentration required for its effect on the ovary. Several investigations have indicated that LH secretion in laboratory rats and mice is extremely sensitive to environmental stress (Dunn et al., 1972; Bronson et al., 1973; Seyler and Reichlin, 1973). An interesting study on young women has shown a suppression of the LH surge upon hospitalization, indicating a stress induced delay of ovulation (Peyser et al., 1973). It may be that LH secretion is suppressed in these female deermice, resulting in a decrease of estrogen production and secretion, and perhaps even inhibiting ovulation. Investigations using antiserum to LH suggest that a decrease of LH in itself can alter estrogen secretion. If anti-LH was injected into cycling rats before 21.00 hours on the day prior to proestrus, estrogen secretion was blocked which prevented the proestrus pituitary LH discharge and ovulation (Schwartz and Gold, 1967; Schwartz and Ely, 1970). In contrast, similar injections of antiserum to FSH in the rat on the day of proestrus, or on the day prior to proestrus, did not prevent secretion of estrogen or ovulation (Schwartz et al., 1973). Also, one cannot exclude the possibility that both FSH and LH are secreted at levels within the control range but that their secretion is out of synchrony, yielding subnormal ovarian function. The foregoing hypotheses emphasize the need for exact measurements of LH

and the estrogens to ascertain the reproductive inhibition mechanisms in population female deermice.

Previous studies showed that nulliparous population females had significantly more atretic follicles than control females (Terman, 1965; Albertson et al., 1975). If ovarian histology among laboratory populations are similar, then an interesting observation can be drawn from these data on FSH. The mechanisms which promote the development of follicular atresia are not well understood (Everett, 1961; Young, 1961). However, in Peromyscus population females, it appears that a reduction in serum FSH is not requisite for development of follicular atresia. Evidently follicular atresia occurs in inhibited female deermice which have FSH levels (Tables 1 and 3) within the control range. The presumed lower estrogen levels in these females may play an important role in follicular atresia development. Direct information on LH and the estrogen levels, along with concomitant ovarian histology studies, should shed further light on this ovarian phenomenon.

Males

The data from this study show that the circulating levels of LH in male deermice from experimental populations are markedly decreased to approximately one-half the level of controls (Table 4, Figure 6). Reduction in testicular androgens is clearly indicated by the observation that the mean paired testis weights of population males is only twenty-four percent of control males (Table 4, Figure 6). Since the mean

relative paired testis weights of population males is significantly smaller than the mean of controls (Table 4), the smaller testes weight condition cannot be solely attributed to the smaller body weight condition found in these animals.

These data suggest that chronic exposure to behavioral variables (which develop intrinsically in a population) is accompanied by a decrease in circulating LH. This alteration in circulating LH levels may be a mechanism whereby reproductive function is curtailed in experimental population male deermice. Since LH is known to stimulate production and secretion of androgens from the Leydig cells in the testes (Hall and Eik-Nes, 1962; El Safoury and Barke, 1974), this reduction in circulating LH could result in lowered androgen secretion. Although direct measurements of the androgen levels were not undertaken in this study, it is possible to assess the androgen status of these male deermice by examining their seminal vesicle (vesicular gland) weights (Moore et al., 1930). A reduction of circulating androgens is reflected in the weights of the seminal vesicles of population males which were only twelve percent of the weight of controls.

Lowered circulating LH and lowered androgen levels could explain, in part, the impairment of spermatogenesis and the testicular regression observed in reproductively inhibited population deermice (Keller, 1974). Several studies have shown that the absence of androgens in adult mammals yields impaired spermatogenesis (Steinberger, 1971). Reduced androgens could also affect the behavior of population males.

Various studies have shown that castration of adult male mice reduces aggressive behavior, and that androgen therapy restores this behavior (Beeman, 1947; Tollman and King, 1956; Bevan et al., 1960). The lowered androgen levels may reduce aggressive behavior towards competing male animals and may even reduce copulatory behavior.

The mechanisms whereby circulating LH is lowered in population males are presently not known. Reduction of LH may be the result of altered metabolism, and/or clearance rate of the hormone from the blood. Depression of the levels of release factor secreted by neurosecretory cells in the hypothalamus could also account for a decrease in circulating LH. A change in the threshold of neural pathways of the hypothalamus due to cues from the external environment may cause altered release patterns of LRF (luteinizing hormone release factor). Then again, it may be that the sensitivity of the hypophysis is altered so that it fails to respond to LRF.

Increased ACTH secretions in response to behavioral variables may play an important role in altering or shifting hypothalamic-hypophysial function. Recent studies indicate that reproductive function in Peromyscus is remarkably sensitive to inhibition by exogenous ACTH and that this inhibitory action is at the level of the pituitary or higher centers (Christian et al., 1965; Pasley and Christian, 1972; Ogle, 1975). A preliminary study on corticosterone levels in population deermice suggests that ACTH may be elevated

in animals from asymptotic populations (Sung, 1974). If elevated ACTH plays a role during curtailed growth, it may do so by inundating receptor sites in the hypophysis blocking the messenger LRF, and thereby, lowering gonadotropin release. An alternative mechanism may be that increased levels of ACTH act directly on the hypothalamus altering thresholds of neural pathways. However, despite the ability of ACTH to inhibit reproduction, it seems more probable that reproductive inhibition in Peromyscus maniculatus is achieved more directly through the CNS (Christian, 1971). Secretion rates of ACTH and gonadotropin may be totally independent of each other. The CNS, in response to external environmental cues, could suppress gonadotropin release while enhancing ACTH secretion. Further investigations addressed specifically to this problem of neural control of gonadotropin release in inhibited male deermice need to be undertaken.

The finding of smaller mean body weights in population males and females (Tables 3 and 4) is different from the body weight findings of an earlier study (Terman, 1965). Possibly differences in the type and size of control cages account for this discrepancy in body weights. However, a recent study by Albertson et al., (1975), concurs with the findings of the present study. Albertson et al. point out that the differences between control and experimental population female body weights may be due to some factors other than an inhibition of total body development. The significant positive regression observed between age and body weights of control

males in the present study (Appendix D) suggests that the male animal becomes heavier in proportion to the period of time spent in a control cage. This increase in weight is most likely the cause of a gradual fat accumulation stemming from the lack of sufficient activity (Albertson et al., 1975). An increase in control deermouse body weight over the duration of the experiment may explain the significant difference found between the control and population groups. However, some inhibition of total body development in population deermice cannot be ruled out. It could be that the levels of growth hormone and other metabolic regulators are altered during the inhibited state, culminating in arrested growth and development.

The significant negative regression between age and seminal vesicle weights of control male animals (Appendix D) suggests that there was a gradual decrease in androgen secretion over the duration of the study. Perhaps control males inure to the fact that their female cage partner is inaccessible, which results in a tapering off of androgen secretion. Whether androgens remain within the reproductive functional range, or whether reduced androgen is climateric, has not been examined. A similar regression was not observed for males in any of the experimental populations (Appendix D). This suggests that inhibition of reproductive function develops very early in the individuals of a population (before 100 days of age). Seemingly, the majority of the population offspring males are in an arrested reproductive state. How-

ever, this arrested condition, probably brought about by decreased levels of LH and androgens, is not irreversible. Terman (1973b; 1975) has found when males are removed from reproductively inhibited populations and paired with population females, that reproductive recovery occurs. Nevertheless, a period of adjustment or maturation, perhaps behavioral as well as physiological, seems to be required before reproduction can occur.

LIST OF APPENDICES

APPENDIX A

Body Weights (gm), Reproductive Organ Weights (mg), and Serum LH Concentrations (ng/ml) of Control Male Deermice in the Two Sacrifice Periods

	Morning Period†		Afternoon Period∞	
	N	Mean ± SE	N	Mean ± SE
Body Weights	(32)	19.1± 0.5	(65)	19.4± 0.5
Testes (paired)	(30)	339.4±15.9	(62)	345.7± 7.9
Vesicular Glands (paired)	(27)	179.0±13.2	(62)	193.5± 7.9
Serum LH	(27)	29.9± 5.5	(42)	35.2± 4.7

† Morning Period (0900-1030 Hrs)
∞ Afternoon Period (1500-1630 Hrs)

APPENDIX B

Body Weights (gm), Reproductive Organ Weights (mg), Relative Reproductive Organ Weights (mg/gm) and Serum LH Concentrations (ng/ml) of Male Deermice in the Individual Experimental Populations

	Population 1		Population 2		Population 3	
	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE
Body Weights	(5)	13.1± 0.6	(19)	13.7± 0.2	(14)	14.2± 0.3
Testes (paired)	(5)	47.6± 5.8	(20)	76.7± 9.9	(14)	125.0±23.1
Relative Testes	(5)	3.6± 0.5	(19)	5.5± 0.7	(14)	8.8± 1.1
Vesicular Glands (paired)	(5)	3.3± 0.6	(21)	13.6± 3.3	(14)	49.5±18.3
Relative Vesicular Glands	(5)	0.2± 0.1	(19)	0.9± 0.2	(14)	3.4± 1.5
Serum LH	(5)	25.9± 5.2	(17)	16.5± 3.7	(14)	14.3± 3.2

APPENDIX C

Pearson Product-Moment Correlation Coefficients
for Control and Population Males

Comparison	N	r	P	Population
Body Weight versus Paired Testis Weight	88	0.270122	0.0106	Control
	5	-0.408041	0.5021	1
	18	0.337383	0.1682	2
	14	0.270122	0.1926	3
Body Weight versus Paired Vesicular Gland Weight	86	0.040271	0.7139	Control
	5	-0.324678	0.5955	1
	19	0.10513	0.6713	2
	14	0.441317	0.1113	3
Paired Testis Weight versus Paired Vesicular Gland Weight	94	0.50036	0.0001	Control
	5	0.937951	0.0166	1
	20	0.799284	0.0001	2
	14	0.9859	0.0001	3
Paired Testis Weight versus Serum LH	62	-0.103388	0.5785	Control
	5	0.05718	0.9245	1
	16	-0.43378	0.8675	2
	14	0.789822	0.0010	3
Paired Vesicular Gland Weight versus Serum LH	62	0.18491	0.1465	Control
	5	0.230308	0.7066	1
	17	-0.145226	0.5840	2
	14	0.687355	0.0066	3

APPENDIX D

Linear Regression Coefficients
for Control and Population Males

Comparison	N	r	P	y = m(x) + b		Population
				m	b	
Age versus Body Weight	89	0.309861	0.0031	0.017952	15.30	Control
	5	0.510876	0.3791	0.013901	10.22	1
	18	-0.269528	0.2794	-0.005017	14.63	2
	14	0.385765	0.1731	0.010573	11.90	3
Age versus Paired Vesicular Gland Weight	89	-0.325470	0.0019	-0.301046	258.09	Control
	5	-0.180565	0.7714	-0.005670	4.49	1
	20	-0.105118	0.1035	-0.028360	19.02	2
	14	0.068844	0.8151	0.098602	27.32	3

APPENDIX E

Body Weights (gm), Reproductive Organ Weights (mg), and Serum FSH Concentrations (ng/ml) in Control Female Deermice at Various Stages of the Estrus Cycle

	DIESTRUS (AM)		DIESTRUS (PM)		PROESTRUS (AM)		PROESTRUS (PM)	
	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE
Body Weights	(7)	14.9± 0.4	(11)	16.1± 0.6	(7)	17.4± 0.7	(13)	17.5± 0.6
Ovaries (paired)	(7)	12.1± 1.8	(11)	13.3± 1.4	(5)	23.5± 4.5	(12)	17.4± 1.4
Uterus	(7)	35.6± 4.9	(11)	38.4± 3.3	(5)	68.9±10.2	(13)	78.3± 9.6
Serum FSH	(8)	79.2±30.7	(10)	105.3±30.3	(7)	152.6±27.7	(11)	263.1±39.1

	ESTRUS (AM)		ESTRUS (PM)		METESTRUS (AM)		METESTRUS (PM)	
	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE
Body Weights	(8)	17.0± 0.4	(8)	16.0± 0.8	(4)	19.1± 0.8	(10)	17.1± 0.7
Ovaries (paired)	(8)	17.1± 2.1	(8)	16.3± 3.2	(5)	16.6± 3.7	(10)	16.0± 1.7
Uterus	(8)	80.9± 9.8	(8)	69.8±18.4	(5)	55.4±20.8	(10)	43.8± 5.1
Serum FSH	(8)	311.1±59.1	(8)	267.2±37.0	(7)	211.3±21.8	(8)	175.2±22.7

APPENDIX F

Body Weights (gm), Reproductive Organ Weights (mg), and Serum FSH Concentrations (ng/ml) in Control Female Deermice During Proestrus Night (2100-2200 Hrs)

	N	Mean ± SE
Body Weights	(5)	16.2± 1.2
Ovaries (paired)	(4)	11.5± 1.2
Uterus	(4)	66.8±11.9
Serum FSH	(5)	328.0±35.1

APPENDIX G

Body Weights (gm), Reproductive Organ Weights (mg), and Serum FSH Concentrations (ng/ml) in Acyclic Control Females

	N	Mean ± SE
Body Weights	(9)	14.9± 0.5
Ovaries (paired)	(9)	11.4± 3.4
Uterus	(9)	23.0± 9.5
Serum FSH	(9)	415.2±83.4

APPENDIX H

Body Weights (gm), Reproductive Organ Weights (mg),
Relative Reproductive Organ Weights (mg/gm) and Serum FSH Concentrations (ng/ml)
of Female Deermice in the Individual Experimental Populations

	Population 1		Population 2		Population 3	
	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE
Body Weights	(11)	13.3± 0.5	(14)	12.3± 0.3	(9)	12.4± 0.4
Ovaries (paired)	(12)	5.4± 0.9	(13)	5.0± 0.5	(9)	4.3± 0.5
Relative Ovaries	(11)	0.4± 0.1	(13)	0.4± 0.1	(9)	0.3± 0.1
Uterus	(12)	7.9± 0.7	(13)	9.4± 2.2	(9)	10.3± 1.6
Relative Uterus	(11)	0.6± 0.1	(13)	0.7± 0.2	(9)	0.8± 0.2
Serum FSH	(9)	363.4±89.6	(9)	230.9±50.8	(8)	216.3±43.2

APPENDIX I

Pearson Product-Moment Correlation Coefficients
for Control and Population Females

	N	r	P	Population
Body Weights versus Paired Ovary Weight	77	0.548562	0.0001	Control
	11	0.164394	0.6330	1
	13	0.468921	0.1032	2
	9	0.608132	0.0804	3
Body Weight versus Uterus Weight	78	0.332763	0.0032	Control
	11	0.050536	0.8581	1
	13	0.439801	0.1299	2
	9	0.776953	0.0136	3
Paired Ovary Weight versus Uterus Weight	76	0.172871	0.1315	Control
	12	0.162331	0.6188	1
	13	0.944576	0.0001	2
	9	0.917814	0.0008	3
Paired Ovary Weight versus Serum FSH	53	0.102952	0.5116	Control
	9	-0.392006	0.2971	1
	7	-0.155742	0.7360	2
	8	-0.645559	0.0822	3
Uterus Weight versus Serum FSH	53	0.117083	0.6486	Control
	9	-0.52045	0.1489	1
	7	-0.474321	0.2823	2
	8	-0.644453	0.0829	3

APPENDIX J

Linear Regression Coefficients
for Control and Population Females

Comparison	N	r	P	y = m(x) + b		Population
				m	b	
Age versus Body Weight	76	0.070070	0.5791	0.002280	16.39	Control
	11	0.017085	0.9602	0.000645	13.24	1
	13	0.067258	0.8272	0.001374	12.11	2
	9	0.493240	0.1773	0.011904	9.96	3
Age versus Paired Ovary Weight	76	0.100756	0.4245	0.009786	14.26	Control
	11	-0.485981	0.1296	-0.035074	12.89	1
	13	0.107209	0.7274	0.003507	4.45	2
	9	0.467529	0.2044	0.014709	1.17	3

BIBLIOGRAPHY

Albertson, B.D., Bradley, E.L., and Terman, C.R. (1975). Plasma progesterone concentrations in prairie deer-mice (Peromyscus maniculatus bairdii), from experimental laboratory populations. J. Reprod. Fert. 42: 407-414.

Ajika, K., Kalra, S.P., Fawcett, C.P., Krulich, L., and McCann, S.M. (1972). The effect of stress and nembutal on plasma levels of gonadotropins and prolactin in ovariectomized rats. Endocrinology 90: 707-715.

Armstrong, D.T. (1968). Hormonal control of uterine lumen fluid retention in the rat. Amer. J. Physiol. 214: 764-771.

Bartke, A. (1966). Influence of prolactin on males fertility in dwarf mice. J. Endocr. 35: 419-420.

Bast, J.D., and Greenwald, G.S. (1974). Serum profiles of follicle-stimulating hormone, luteinizing hormone, and prolactin during the estrous cycle of the hamster. Endocrinology 94: 1295-1299.

Beeman, E.A. (1947). The effect of male hormone on aggressive behavior in mice. Physiol. Zool. 20: 373-405.

Berson, S.A., and Yalow, R.S. (1959). Quantitative aspects of the reaction between insulin and insulin-binding antibody. J. Clin. Invest. 38: 1996-2016.

Bevan, W., Levy, G.W., Whitehouse, J.M., and Bevan, J.M. (1957). Spontaneous aggressiveness in two strains of mice castrated and treated with one of three androgens. Physiol. Zool. 30: 341-349.

Bevan, W., Davies, W.F., and Levy, G.W. (1960). The relation of castration, androgen therapy and pre-test fighting experience to competitive aggression in C57BL/10 mice. An. Beh. 8: 6-12.

Bex, F.J., and Goldman, B.D. (1975). Serum gonadotropins and follicular development in the syrian hamster. Endocrinology 96: 928-933.

Bingel, A.S., and Schwartz, N.B. (1969). Pituitary LH content and reproductive tract changes during the mouse oestrous cycle. J. Reprod. Fert. 19: 215-222.

Blake, C.A., Norman, R.L., and Sawyer, C.H. (1973). Validation of an ovine-ovine LH radioimmunoassay for use in the hamster. Biol. Reprod. 8: 299-305.

Bogdanove, E.M. (1963). Direct gonad-pituitary feedback: an analysis of effects of intracranial estrogenic depots on gonadotrophin secretion. Endocrinology 73: 696-712.

Bronson, F.H., Stetson, M.H., and Stiff, M.E. (1973). Serum FSH and LH in male mice following aggressive/non-aggressive interaction. Physiol. Behav. 10: 369-372.

Butcher, R.L., Collins, W.E., and Fugo, N.W. (1974). Plasma concentrations of LH, FSH, prolactin, progesterone and estradiol-17β throughout the 4-day estrous cycle of the rat. Endocrinology 94: 1704-1708.

Christian, J.J. (1971). Population density and reproduction efficiency. Biol. Reprod. 4: 248-294.

Christian, J.J., Lloyd, J.A., and Davis, D.E. (1965). The role of endocrines in the self-regulation of mammalian populations. Rec. Prog. Hor. Res. 21: 501-578.

Clark, F.H. (1936). The estrous cycle of the deer-mouse, Peromyscus maniculatus. Contrib. Lab. Vert. Genetics, Univ. Mich., No. 1, 8 pp.

Conover, W.J. (1971). Practical Nonparametric Statistics. New York: John Wiley and Sons, Inc.

Cowie, A.T. (1966). Anterior pituitary function in lactation. In: The Pituitary Gland. 412-443. Berkley: Univ. of Calif. Press. Vol. 2.

Daane, T.A., and Parlow, A.F. (1971). Periovulatory patterns of rat serum follicle stimulating hormone and luteinizing hormone during the normal estrous cycle: effects of pentobarbital. Endocrinology 88: 653-663.

Desjardins, C., Chapman, V.M., and Bronson, F.H. (1970). Hypophysial LH and FSH release and uterine nucleic acid changes during the mouse estrous cycle. Anac. Rec. 167: 465-471.

Diebel, N.D., Yamamoto, M., and Bogdanove, E.M. (1973). Discrepancies between radioimmunoassays and bioassay for rat FSH: evidence that androgen treatment and with-

drawal can alter bioassay-immunoassay ratios. Endocrinology 92: 1065-1078.

Duddleson, W.G., Midgley, A.R., Jr., and Niswender, G.D. (1972). Computer program sequence for analysis and summary of radioimmunoassay data. Comput. Biomed. Res. 5: 205-217.

Dunn, J.D., Arimura, A., and Scheving, L.E. (1972). Effect of stress on circadian periodicity in serum LH and prolactin concentration. Endocrinology 90: 29-33.

El Safoury, S., and Bartke, A. (1974). Effects of follicle-stimulating hormone and luteinizing hormone on plasma testosterone levels in hypophysectomized and in intact immature and adult male rats. J. Endocr. 61: 193-198.

Euker, J.S., Meites, J., and Riegle, G.D. (1975). Effects of acute stress on serum LH and prolactin in intact, castrate and dexamethasone-treated male rats. Endocrinology 96: 85-92.

Everett, J.W. (1961). The mammalian female reproductive cycle and its controlling mechanisms. In: Sex and Internal Secretions. 497-555. Young, W.C., ed. Baltimore: Williams and Wilkins. Vol. 1.

Gay, V.L. (1972). The hypothalamus: physiology and clinical use of releasing factors. J. Reprod. Fert. 23: 50-63.

Gay, V.L., Midgley, A.R., and Niswender, G.D. (1970). Patterns of gonadotropin secretion associated with ovulation. Fed. Proc. 29: 1880-1887.

Goldman, B.D., and Porter, J.C. (1970). Serum LH levels in intact and castrated golden hamsters. Endocrinology 87: 676-679.

Greenwood, F.C., Hunter, W.M., and Glover, J.S. (1963). The preparation of ^{131}I-labelled growth hormone of high specific radioactivity. Biochem. J. 89: 114-123.

Greep, R.O., Van Dyke, H.B., and Chow, B.F. (1942). Gonadotropins of the swine pituitary. Endocrinology 30: 635-649.

Greep, R.O. (1961). Physiology of the anterior hypophysis in relation to reproduction. In: Sex and Internal Secretions. 240-301. Young, W.C., ed. Baltimore: Williams and Wilkins. Vol. 1.

Hall, P.F., and Eik-Nes, K.B. (1962). Action of gonadotropic hormones upon rabbit testis in vitro. Biochim. biophys. Acta 63: 411-422.

Keller, S.R. (1974). Ultrastructure of the seminiferous epithelium of prairie deermouse (Peromyscus maniculatus bairdii), from a freely-growing, confined population. M.A. Thesis, College of William and Mary. 78 pp.

Kirton, K.T., and Hafs, H.D. (1965). Sperm capacitation by uterine fluid or beta-amylase in vitro. Science 150: 618-619.

Kovacic, N., and Parlow, A.F. (1972). Alterations in serum FSH/LH ratios in relation to the estrous cycle, pseudopregnancy, and gonadectomy in the mouse. Endocrinology 91: 910-915.

Labhsetwar, A.P., Joshi, H.S., and Watson, D. (1973). Temporal relationship between estradiol, estrone and progesterone secretion in the ovarian venous blood and LH in the peripheral plasma or cyclic hamsters. Biol. Reprod. 8: 321-326.

Leonard, S.L. (1950). The reduction of uterine sperm and uterine fluid on fertilization of rat ova. Anat. Rec. 106: 607-615.

Lloyd, C.W., Rogers, W.F., and Williams, R.H. (1946). Quantitative studies of the opening of the vagina of immature rats following injections of female blood and urine. Endocrinology 39: 256-260.

Lostroh, A.J., and Johnson, R.E. (1966). Amounts of interstitial cell-stimulating hormone and follicle-stimulating hormone required for follicular development, uterine growth, and ovulation in the hypophysectomized rat. Endocrinology 79: 991-996.

MacDonald, G.J. (1971). Effect of FSH, prolactin, and LH on serum progesterone levels in the cycling primate. Fed. Proc. 30: 309.

McCann, S.M. (1971). Hypophysiotropic hormones of the hypothalamus. In: The Neuroendocrinology of Human Reproduction. 91-109. Mack, H.C., and Sherman, A.I., eds. Springfield, Illinois: Charles C. Thomas.

Midgley, A.R., Jr., Rebar, R.W., and Niswender, G.D. (1969). Radioimmunoassays employing double antibody techniques. In: 1st Karolinska Symposium on Research Methods in Reproductive Endocrinology-Immunoassay of Gonado-

trophins. 247-256. Diczflausy, E., ed. Copenhagen: Periodka.

Midgley, A.R., Jr., Niswender, G.D., Gay, V.L., and Reichert, L.E., Jr. (1971). Use of antibodies for characterization of gonadotropins and steroids. Rec. Prog. Hor. Res. 27: 235-301.

Moore, C.R., Hughes, W., and Gallagher, T.F. (1930). Rat seminal-vesicle cytology as a testis-hormone indicator and the prevention of castration changes by testis extract injection. Am. J. Anat. 45: 109-136.

Murr, S.M., Geschwind, I.I., and Bradford, G.E. (1973). Plasma LH and FSH during different oestrous cycle conditions in mice. J. Reprod. Fert. 32: 221-230.

Neill, J.D. (1970). Effect of "stress" on serum prolactin and luteinizing hormone levels during the estrous cycle of the rat. Endocrinology 87: 1192-1197.

Nequin, L., and Schwartz, N.B. (1973). Hormonal studies of the four- and five-day rat cycle. Biol. Reprod. 9: 75.

Niswender, G.D., Reichert, L.E., Jr., Midgley, A.R., Jr., and Nalbandov, A.V. (1969). Radioimmunoassay for bovine and ovine luteinizing hormone. Endocrinology 84: 1166-1173.

Noyes, R.W. (1953). The fertilizing capacity of spermatozoa. West J. Surg. Obstet. Gynecol. 61: 342-349.

Odell, W.D., and Moyer, D.L. (1971). Physiology of Reproduction. Saint Louis: C. V. Mosby Company.

Ogle, T.F. (1974). Effects of ACTH on ovarian histochemistry and maintenance of pregnancy in deermice. Biol. Reprod. 11: 288-296.

Parlow, A.F. (1961). Bioassay of pituitary luteinizing hormone by depletion of ovarian ascorbic acid. In: Human Pituitary Gonadotropins. 300-310. Albert, A., ed. Springfield, Illnois: C. C. Thomas.

Pasley, J.N., and Christian, J.J. (1972). The effect of ACTH, group caging, and adrenalectomy in Peromyscus leucopus with emphasis on suppression of reproductive function. Proc. Soc. Exp. Biol. Med. 139: 921-925.

Peyser, M.R., Ayalon, D., Harell, A., Toaff, R., and Cordova, T. (1973). Stress induced delay of ovulation. Obstet. Gynecol. 42: 667-671.

Ross, G.T., and Vande Wiele, R.L. (1974). The Ovaries. In: Textbook of Endocrinology. 368-422. Williams, R.H., ed. Philadelphia: W. B. Saunders Company.

Schwartz, N.B. (1964). Acute effects of ovariectomy on pituitary LH, uterine weight, and vaginal cornification. Am. J. Physiol. 207: 1251-1259.

Schwartz, N.B. (1969). A model for the regulation of ovulation in the rat. Rec. Progr. Horm. Res. 25: 1-55.

Schwartz, N.B., and Gold, J.J. (1967). Effect of a single dose of anti-LH serum at proestrus on the rat estrous cycle. Anat. Rec. 157: 137-150.

Schwartz, N.B., and Ely, C.A. (1970). Comparison of effects of hypophysectomy, antiserum to ovine LH, and ovariectomy on estrogen secretion during the rat estrous cycle. Endocrinology 86: 1420-1435.

Schwartz, N.B., and McCormack, C.A. (1972). Reproduction: gonadal function and its regulation. Ann. Rev. Physiol. 34: 425-472.

Schwartz, N.B., Krone, K., Talley, W.L., and Ely, C.A. (1973). Administration of antiserum to ovine FSH in the female rat: failure to influence immediate events of cycle. Endocrinology 92: 1165-1174.

Searle, S.R. (1971). Linear Models. New York: John Wiley and Sons, Inc.

Selye, H. (1950). Stress. Montreal: Acta, Inc., Medical Publishers.

Seyler, L.E., Jr., and Reichlin, S. (1973). Luteinizing hormone release in the rat induced by blood volume depletion. Endocrinology 92: 295-302.

Sinha, Y.N., Selby, F.W., Lewis, U.J., and Vanderlaan, W.P. (1972). Studies of prolactin secretion in mice by a homologous radioimmunoassay. Endocrinology 91: 1045-1053.

Snedcor, G.W., and Cochran, W.G. (1967). Statistical Methods. Ames, Iowa: The Iowa State Univ. Press.

Steel, R.D.G., and Torrie, J.H. (1960). Principles and Procedures of Statistics. New York: McGraw-Hill Book Co., Inc.

Steelman, S.L., and Pohley, F.M. (1953). Assay of the follicle stimulating hormone based on augmentation with human chorionic gonadotropin. Endocrinology 53: 604-616.

Steinberger, E. (1971). Hormonal control of mammalian spermatogenesis. Physiol. Rev. 51: 1-22.

Stevens, V.C. (1972). Hypophyseal-ovarian interrelationships in humans: endocrine aspects. In: Reproductive Biology. 115-139. Balin, H., and Glasser, S., eds. Amsterdam: Excerpta Medica.

Sung, P. (1974). Personal communication.

Taya, K., and Igarashi, M. (1973). Changes in FSH, LH and prolactin secretion during estrous cycle in rats. Endocrinol. Japon. 20: 199-205.

Terbel, J., Blake, C.A., and Sawyer, C.H. (1972). Serum prolactin levels in lactating rats after suckling or exposure to ether. Endocrinology 91: 49-53.

Terman, C.R. (1965). A study of population growth and control exhibited in the laboratory by prairie deermice. Ecology 46: 890-895.

Terman, C.R. (1969). Weights of selected organs of deermice from asymptotic laboratory populations. J. Mammal. 50: 311-320.

Terman, C.R. (1973a). Recovery of reproductive function by prairie deermice (Peromyscus maniculatus bairdii), from asymptotic populations. An. Beh. 21: 443-448.

Terman, C.R. (1973b). Reproductive inhibition in asymptotic populations of prairie deermice. J. Reprod. Fert., Suppl. 19: 457-463.

Terman, C.R. (1974). Behavior factors associated with cessation of growth of laboratory populations of prairie deermice. Res. Pop. Ecol. 15: 138-147.

Terman, C.R. (1975). Laboratory studies of population regulation in prairie deermice. Proc. Cent. Symp. Sci. Res. (in Press).

Tollman, J., and King, J.A. (1956). The effects of testosterone propionate on aggression in male and female C57BL/10 mice. Brit. J. An. Beh. 4: 147-149.

Varavudhiz, P., and Meites, J. (1974). Serum LH, FSH, and prolactin levels during the estrous cycle in the hamster. Proc. Soc. Exp. Biol. Med. 145: 571-573.

Villee, D.B. (1975). Maturation: sexual. In: Human Endocrinology: A Developmental Approach. 351-398. Philadelphia: W. B. Saunders Company.

Warren, M.R. (1936). Observations on the uterine fluid of the rat. Am. J. Physiol. 122: 602-608.

White, W.D., and Browning, H.C. (1962). Evidence for the periodic release of luteotropin during the estrous cycle of the mouse. Texas Rep. Biol. Med. 20: 484-493.

Wuttke, W., and Meites, J. (1970). Effects of ether and pentobarbital on serum prolactin and LH levels in proestrus rats. Proc. Soc. Exp. Biol. Med. 135: 648-652.

Young, W.C. (1961). The mammalian ovary. In: Sex and Internal Secretions. 449-496. Young, W.C., ed. Baltimore: Williams and Wilkins. Vol. 1.

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